

Lipid-polymer hybrid nanoparticles loaded with N-acetylcysteine for the modulation of neuroinflammatory biomarkers in Human iPSC-Derived PSEN2 (N141I) Astrocytes as a model of Alzheimer's Disease

Alondra Vargas-Barona,^a Johanna Bernáldez-Sarabia,^a and Ana B. Castro-Ceseña^{a,b*}

a. Departamento de Innovación Biomédica, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Carretera Ensenada- Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, Baja California, Mexico. E-mail: acastro@cicese.mx.

b. CONAHCYT-Departamento de Innovación Biomédica, Centro de Investigación Científica y de Educación Superior de Ensenada, Baja California (CICESE), Carretera Ensenada-Tijuana No. 3918, Zona Playitas, C.P. 22860, Ensenada, Baja California, Mexico.

Supplementary data

Figures

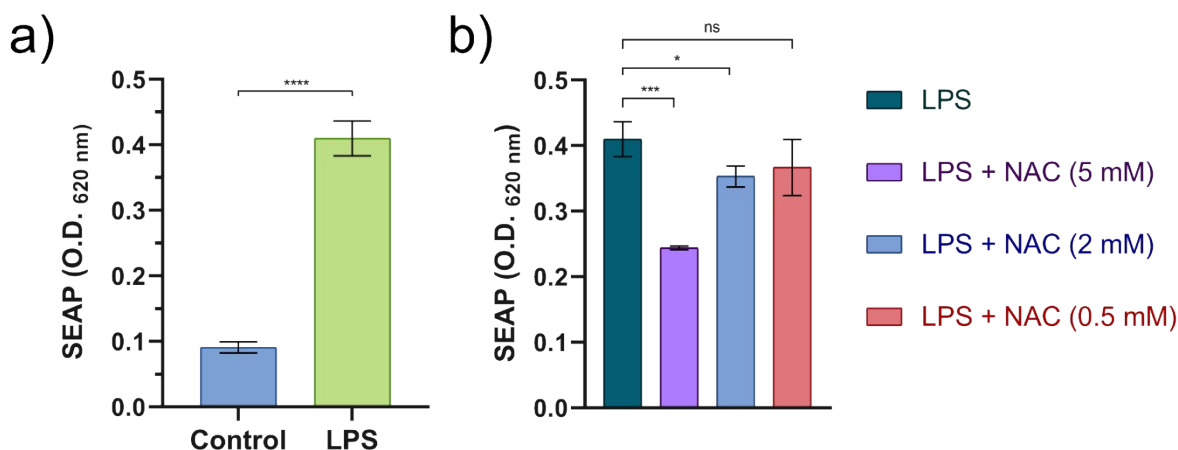


Figure S1. Secreted embryonic alkaline phosphatase (SEAP) activity detected by QUANTI-Blue™. a) SEAP detection from CCD-1112Sk human fibroblasts stimulated with LPS (1 ng/mL) for 24 h, b) Effect of different concentrations of NAC for the inhibition of SEAP production in human fibroblasts. Vertical bars represent the standard deviation of the mean (\pm), $n = 3$ per group. Two-tailed Student's t analysis with $p < 0.05$.

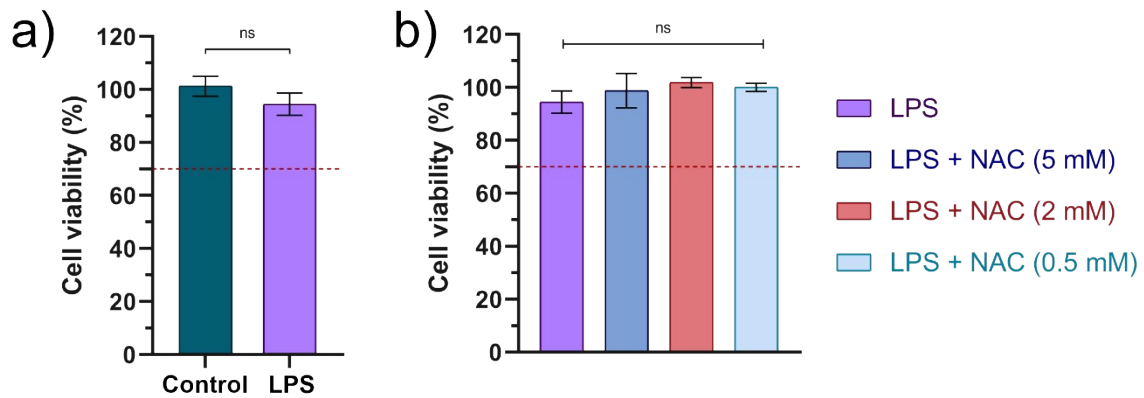


Figure S2. Cell viability in an inflammation model with CCD-1112Sk human fibroblasts stimulated with LPS. a) Percentages of cell viability after stimulation with LPS (1 ng/mL) for 24 h, b) cell viability after stimulation and the application of different concentration of free NAC. Vertical bars represent the standard deviation of the mean (\pm), $n = 3$ per group. One-way ANOVA using Tukey's post hoc analysis with $p < 0.05$.

Tables

Table S1. Primer sequences and characteristics of genes to be evaluated by qPCR in iPSC HA-PSEN2.

Gen	Gene ID	Sequence	Amplicon length (bp)	Reference
GFAP	2670	F: TGGGAGCTTGATTCTCAGCA	122	(Joe, Jeong & Cho, 2015)
		R: CCTGGGCTTGACCTCTCTGTA		
S100 β	6285	F: GGAGACGGCGAATGTGACTT	72	(Jung, 2016)
		R: GAACTCGTGGCAGGCAGTAGTAA		
IL-1 β	3553	F: CTCTGCCTCTTTGTGTGTATGC	74	(Adamik et al., 2013)
		R: GAGGGAAGGAGAGGGAGAGA		
IL-6	3569	F: GGAGACTTGCCTGGTGA AAA	99	(Robinson et al., 2020)
		R: CTGGCTTGTTCCTCACTACTC		
APP	351	F: TGTGTGCTCTCCAGGTCTA	80	(Varhaug et al., 2017)
		R: CAGTTCTGGATGGTCACTGG		
GAPDH	2597	F: CTCCTCCACCTTTGACGCTG	206	(Mathew et al., 2018)
		R: ACCACCCTGTTGCTGTAGCC		

Joe, I.-S., Jeong, S.-G., & Cho, G.-W. (2015). Resveratrol-induced SIRT1 activation promotes neuronal differentiation of human bone marrow mesenchymal stem cells. *Neuroscience Letters*, 584, 97–102. <https://doi.org/10.1016/j.neulet.2014.10.024>.

Jung, N., Park, S., Choi, Y., Park, J.-W., Hong, Y., Park, H., & Jung, S.-C. (2016). Tonsil-Derived Mesenchymal Stem Cells Differentiate into a Schwann Cell Phenotype and Promote Peripheral Nerve Regeneration. *International Journal of Molecular Sciences*, 17(11), 1867. <https://doi.org/10.3390/ijms17111867>.

Adamik, J., Wang, K. Z. Q., Unlu, S., Su, A.-J. A., Tannahill, G. M., Galson, D. L., O'Neill, L. A., & Auron, P. E. (2013). Distinct Mechanisms for Induction and Tolerance Regulate the Immediate Early Genes Encoding Interleukin 1 and Tumor Necrosis Factor α . *PLOS ONE*, 8(8), e70622. <https://doi.org/10.1371/journal.pone.0070622>.

Robinson, K. F., Narasipura, S. D., Wallace, J., Ritz, E. M., & Al-Harhi, L. (2020). -Catenin and TCFs/LEF signaling discordantly regulate IL-6 expression in astrocytes. *Cell Communication and Signaling*, 18(1). <https://doi.org/10.1186/s12964-020-00565-2>.

Varhaug, K. N., Vedeler, C. A., Myhr, K.-M., Aarseth, J. H., Tzoulis, C., & Bindo^e, L. A. (2017). Increased levels of cell-free mitochondrial DNA in the cerebrospinal fluid of patients with multiple sclerosis. *Mitochondrion*, 34, 32–35. <https://doi.org/10.1016/j.mito.2016.12.003>.

Mathew, N. R., Baumgartner, F., Braun, L., O'Sullivan, D., Thomas, S., Waterhouse, M., Müller, T. A., Hanke, K., Taromi, S., Apostolova, P., Illert, A. L., Melchinger, W., Duquesne, S., Schmitt-Grae^e, A., Osswald, L., Yan, K.-L., Weber, A., Tugues, S., ... , & Zeiser, R. (2018). Sorafenib promotes graft- versus-leukemia activity in mice and humans through IL-15 production in FLT3-ITD-mutant leukemia cells. *Nature Medicine*, 24(3), 282–291. <https://doi.org/10.1038/nm.4484>.